

Figure 1. Enzymatic generation of DNA insert encoding a p53-specific shRNA

1/ Anneal stem loop

Primer binding site – sal I RE – U – p53 – stem – lo
 stem – op

2/ Self prime extension (97 bp)

Primer binding site – sal I RE – U – p53 – stem – lo
 Primer binding site – sal I RE – A – p53 – stem – op

3/ UNG and Piperidine Treatment (78 bp)

p53 – stem – lo
 Primer binding site – sal I RE – A – p53 – stem – op

4/ Anneal Primer

Primer binding site – sal I RE p53 – stem – lo
 Primer binding site – sal I RE – A – p53 – stem – op

5/ Second Strand Synthesis (78 bp) and polishing

Primer binding site – sal I RE – T – p53 – stem – loop – stem – p53
 Primer binding site – sal I RE – A – p53 – stem – loop – stem – p53

6/ Sal Digestion (65 bp)

TCGAC – T – p53 – stem – loop – stem – p53
 G – A – p53 – stem – loop – stem – p53

Figure 2. Enzymatic generation of DNA insert encoding a random shRNA

1/ Anneal stem loop

Primer binding site – sal I RE – U – N19 – stem – lo
 stem – op

2/ Self prime extension (97 bp)

Primer binding site – sal I RE – U – N19 – stem – lo
 Primer binding site – sal I RE – A – As19 – stem – op

3/ UNG and Piperidine Treatment (78 bp)

N19 – stem – lo
 Primer binding site – sal I RE – A – As19 – stem – op

4/ Anneal Primer

Primer binding site – sal I RE N19 – stem – lo
 Primer binding site – sal I RE – A – As19 – stem – op

5/ Second Strand Synthesis (78 bp) and polishing

Primer binding site – sal I RE –T– N19 – stem – loop – stem – As19
 Primer binding site – sal I RE –A– N_c19 – stem – loop – stem – As_c19

6/ Sal Digestion (65 bp)

TCGAC –T– N19 – stem – loop – stem – As19
 G –A– N_c19 – stem – loop – stem – As_c19

Figure 3. Suppression of dEGFP-mediated cell fluorescence using a EGFP-specific shRNA expression plasmid

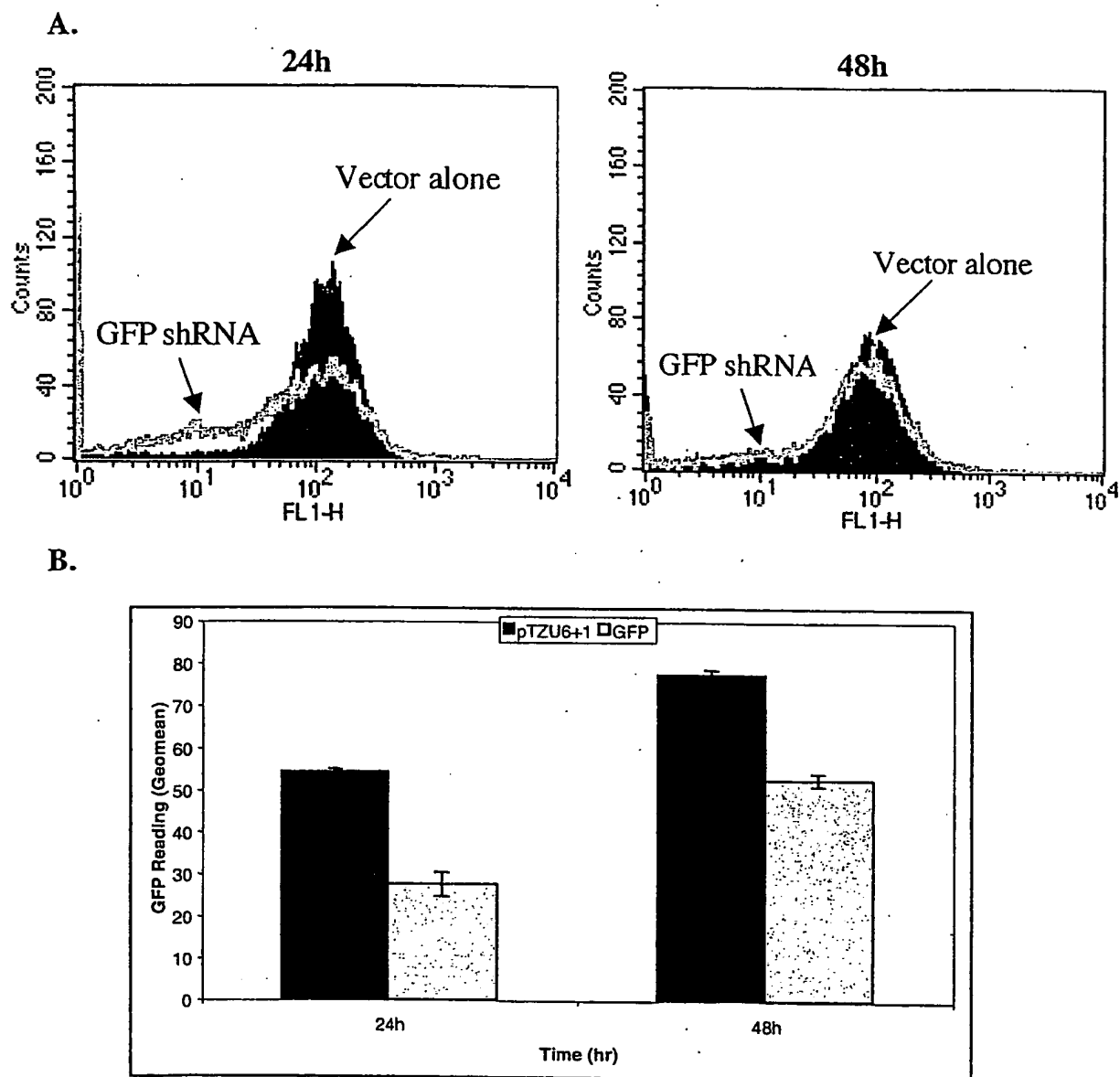


Figure 4. Construction of random shRNA expression library in a modified pLXSN retroviral vector

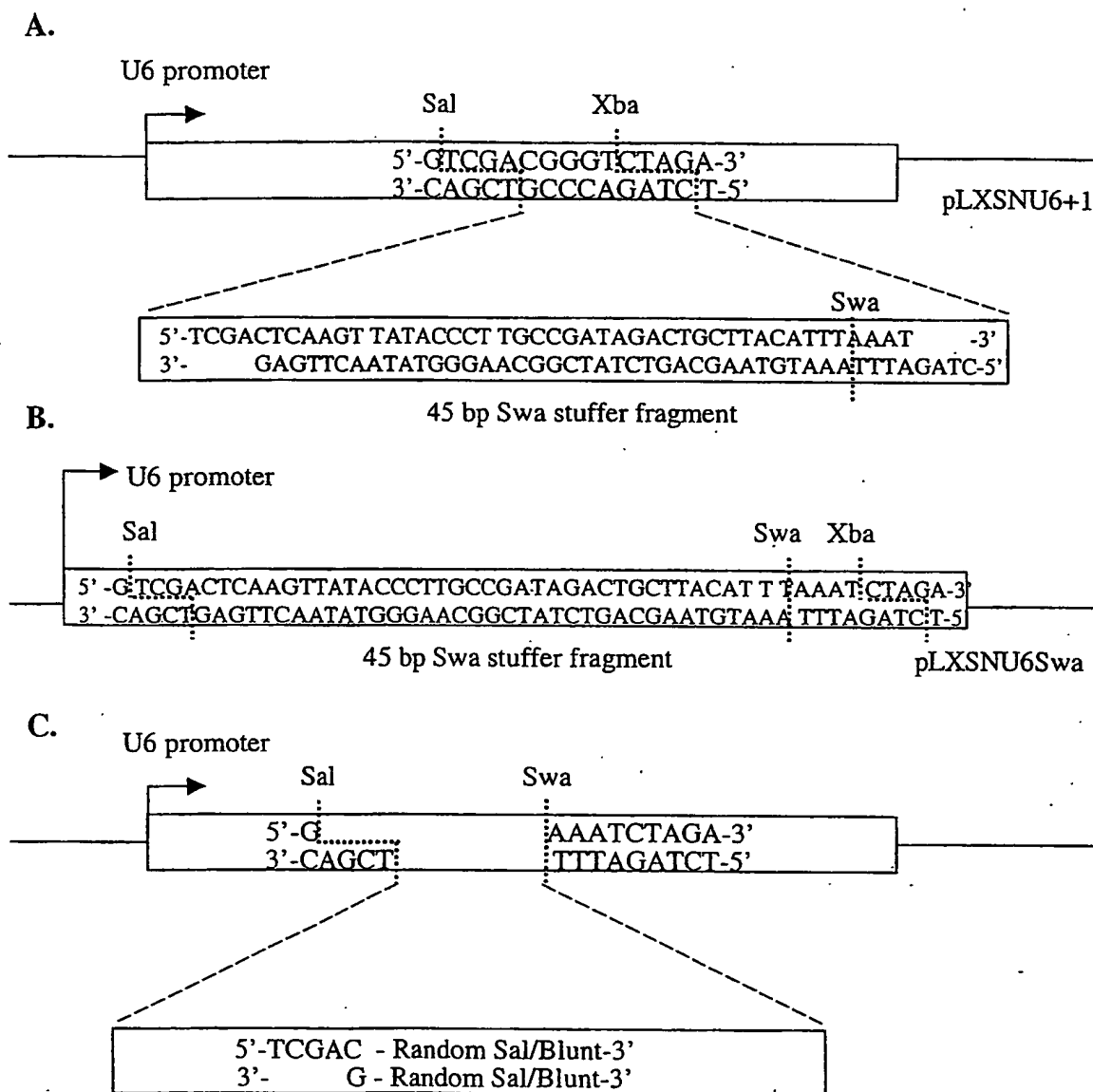


Figure 5. Enzymatic generation of DNA insert encoding complementary sense and antisense RNAs specific for p53.

p53 siRNA Template (63 mer)

5'-CGGTGATTCCGTCGACCAAAAAGACTCCAGTGGTAATCTACTTTTTCTAGAGGTAACAGGCGC-3'

primer (17 mer) 5'-GCGCCTGTACCTCTAG-3'

1/ Anneal Primer to p53siRNA

5'-CGGTGATTCCGTCGACCAAAAAGACTCCAGTGGTAATCTACTTTTTCTAGAGGTAACAGGCGC-3'
3'-GATCTCCATTGTCCGCG-5'

2/ Second Strand Synthesis

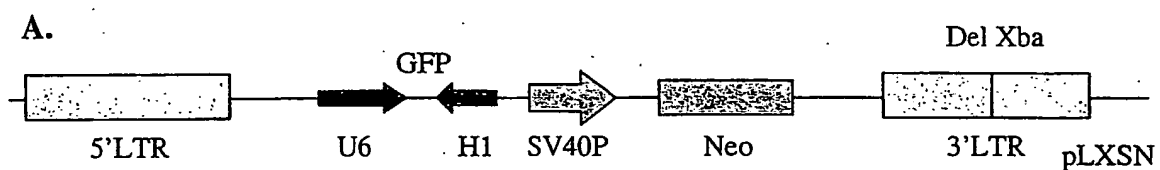
5'-CGGTGATTCCGTCGACCAAAAAGACTCCAGTGGTAATCTACTTTTTCTAGAGGTAACAGGCGC-3'
3'-GCCACTAAGGGAGCTCGTTTT TCTGAGGTCACCATTAGATGAAAAAGATCTCCATTGTCCGCG-5'

3/ SalI and XbaI Digestion

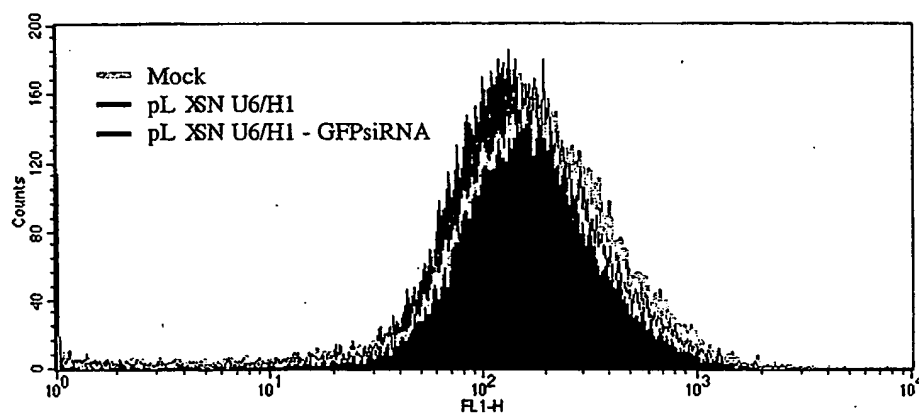
TCGACCAAAAAGACTCCAGTGGTAATCTACTTTTT
GGTTT TTCTGAGGTCACCATTAGATGAAAAAGATC

4/ Dephosphorylate and Purify

Figure 6. Reduction in dEGFP-mediated cell fluorescence in cells transiently infected with a retroviral expression vector encoding EGFP siRNA.



B.



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Figure 7. Reduction in p53 protein levels in HCT116 colon carcinoma cells infected with a retroviral expression vector encoding p53 siRNA.

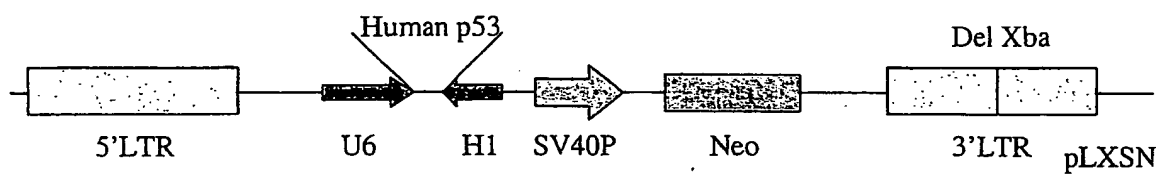
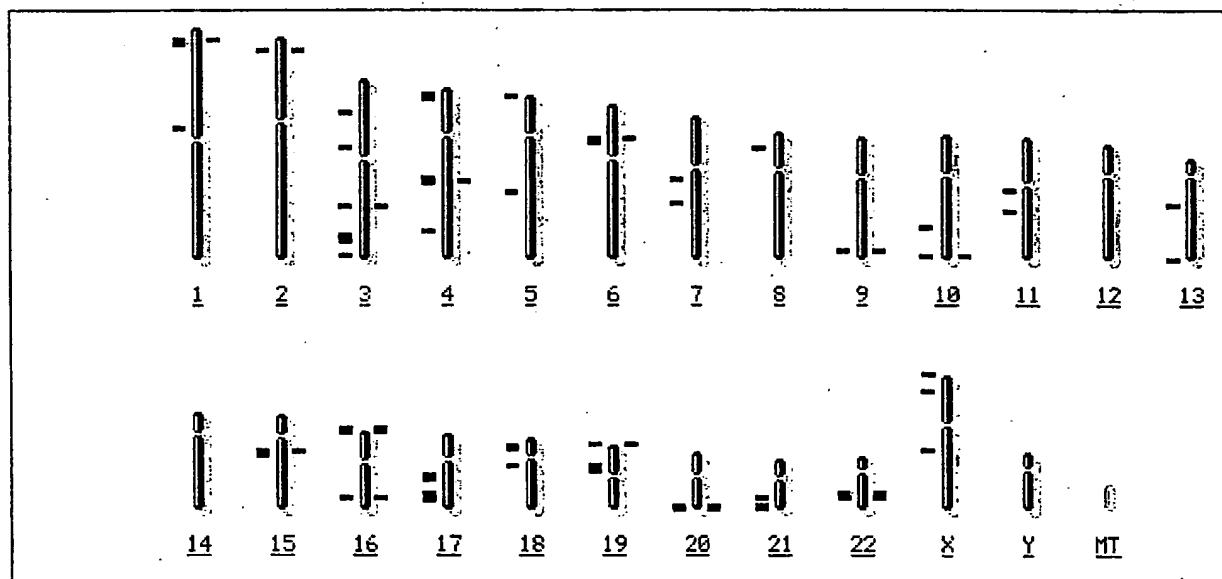


Figure 8. Construction of a genome-wide siRNA retroviral expression library.

C.



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Figure 9. Strategy for generating intracellular siRNAs and effect of the expressed siRNAs on transgene expression.

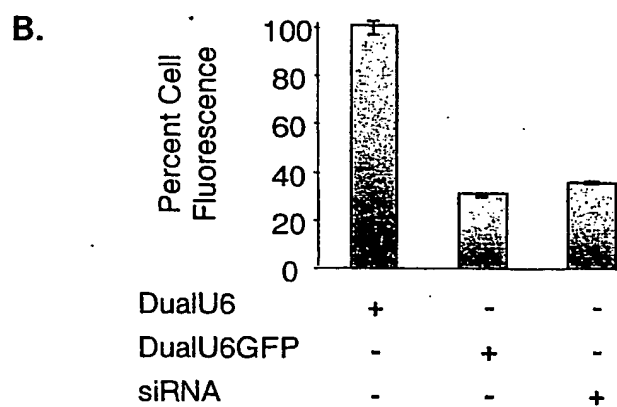
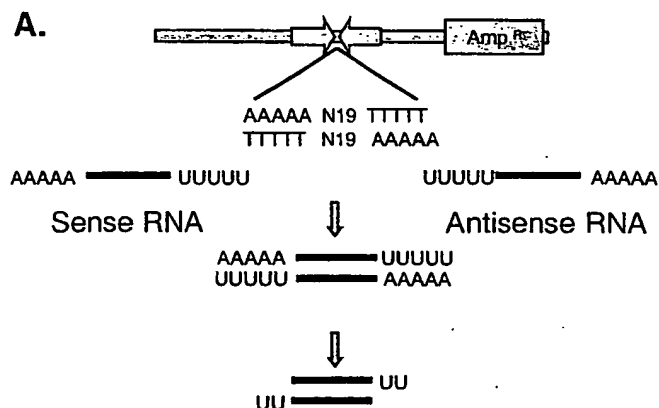
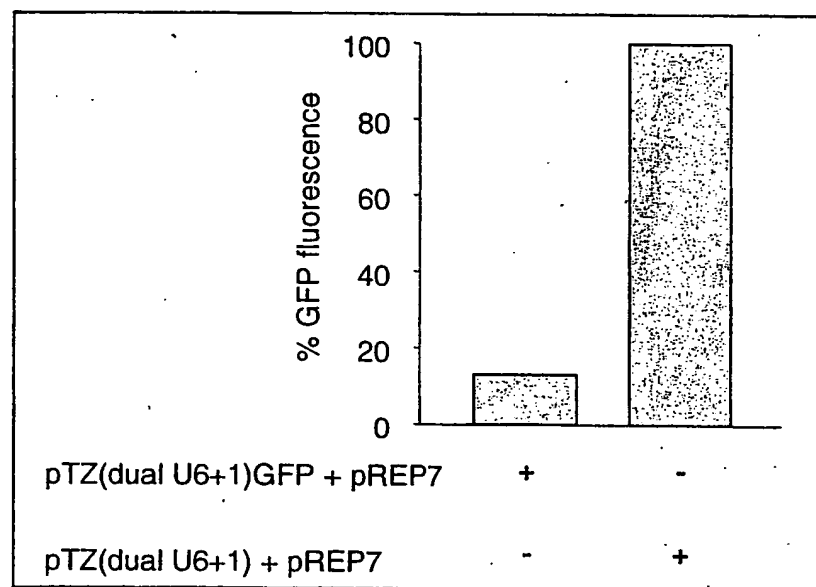


Figure 10. Suppression of dEGFP transgene expression using a stably integrated convergent transcription vector.



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Figure 11. Suppression of target gene expression by the DualU6GFP vector requires the co-expression of both sense and antisense RNAs.

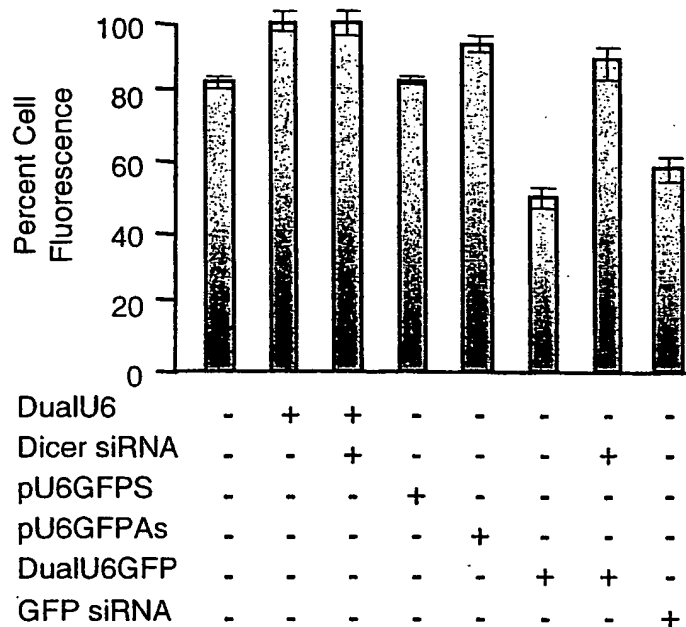
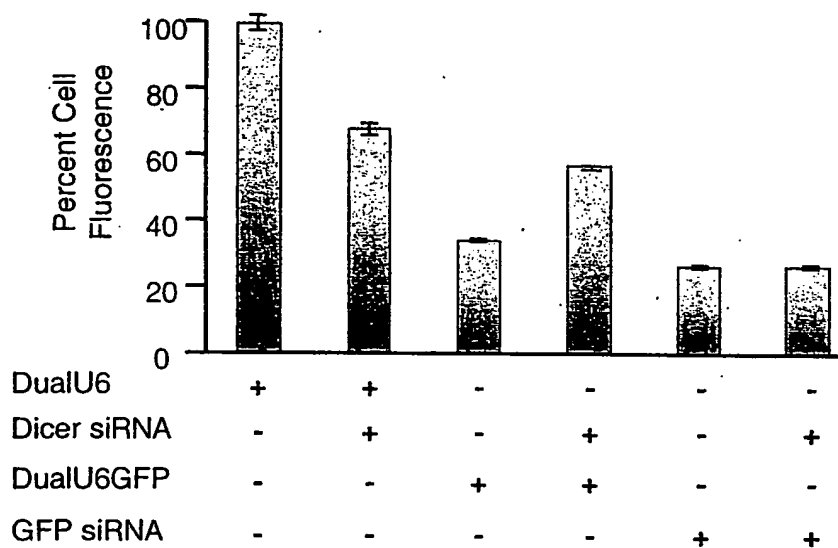


Figure 12. The DualU6GFP expression vector reduces dEGFP target gene expression in a Dicer-dependent manner.



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Figure 13. 5-FU-induced apoptosis in HCT116 cells containing pLXSNU6/H1p53.

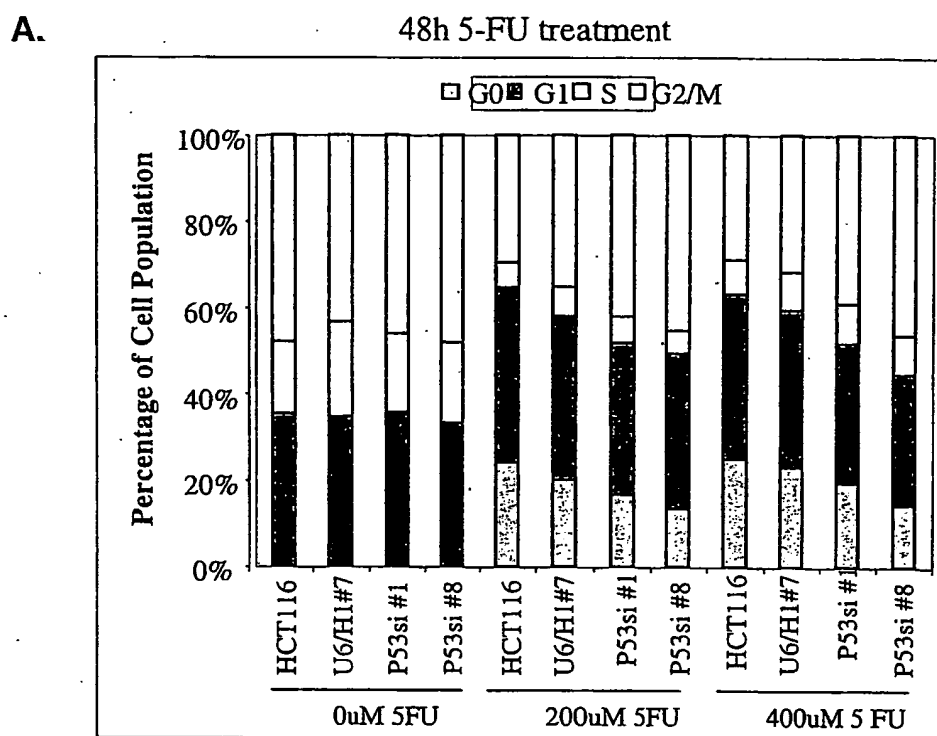
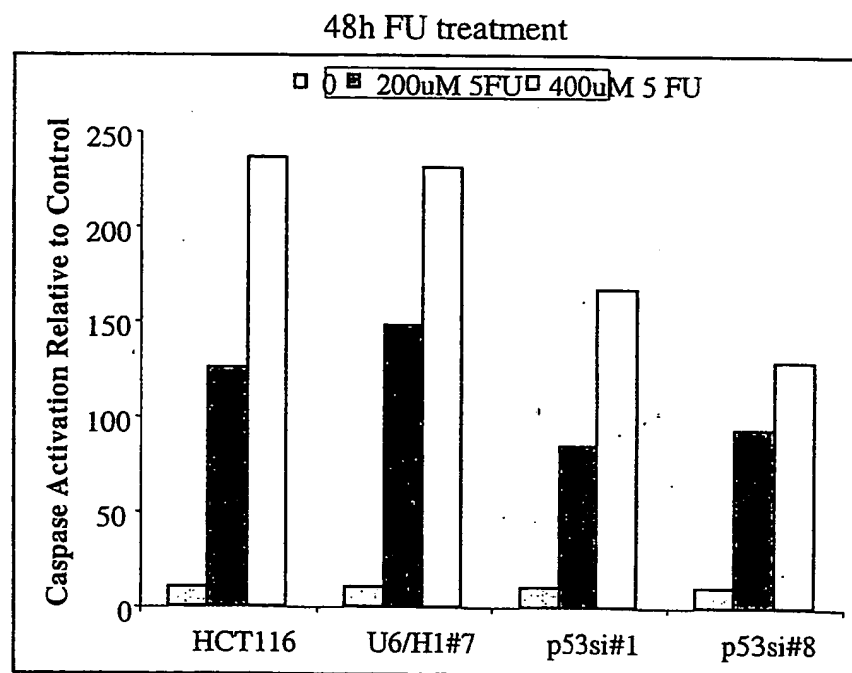


Figure 13. 5-FU-induced apoptosis in HCT116 cells containing pLXSNU6/H1p53.

B.

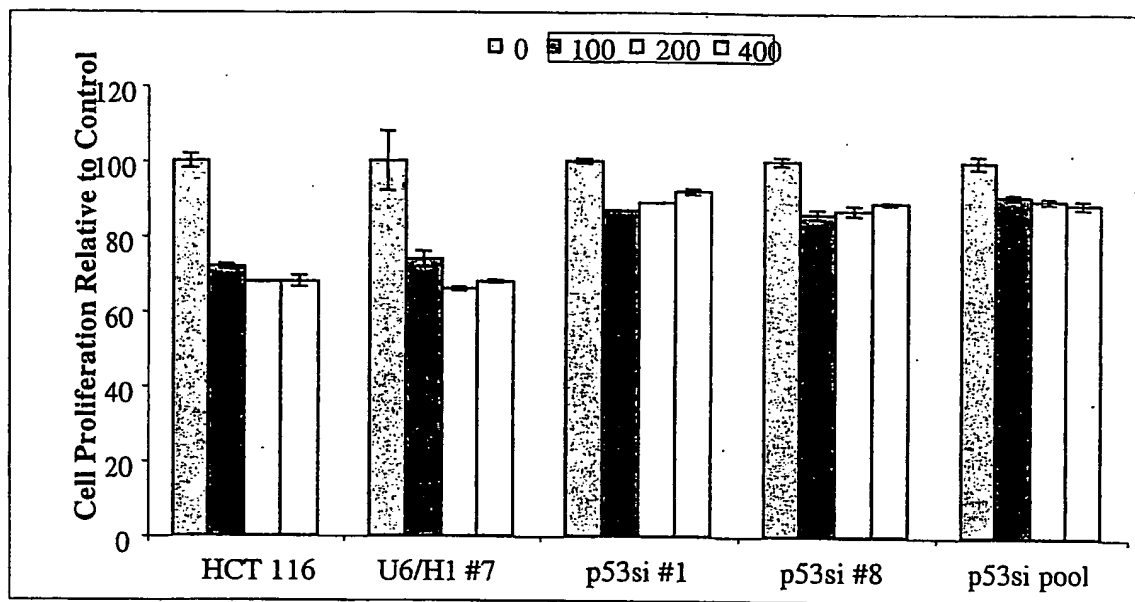


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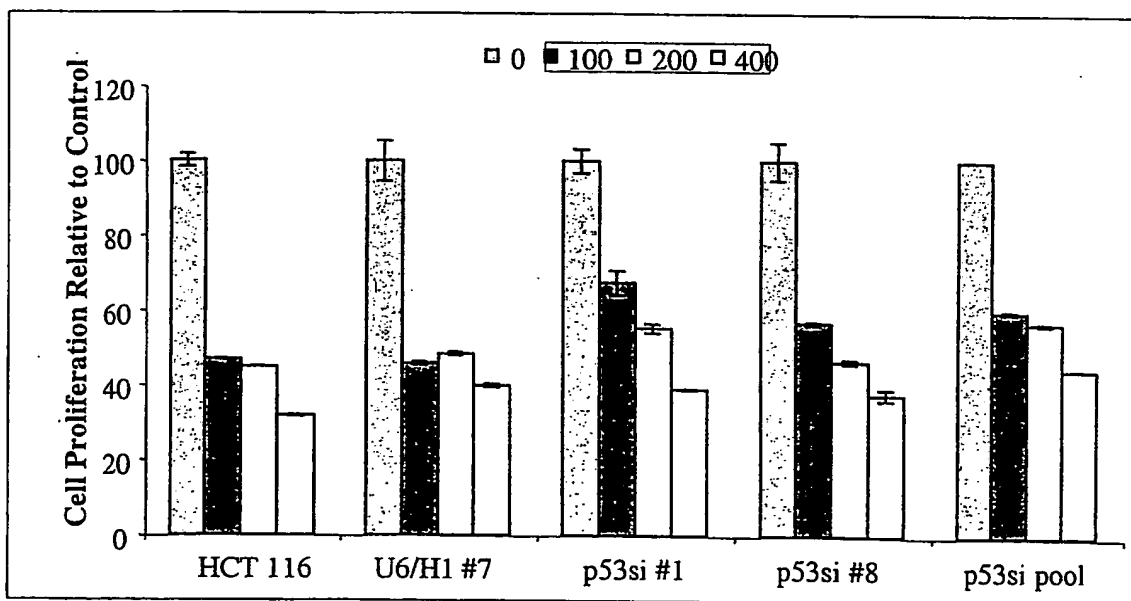
Figure 13. 5-FU-induced apoptosis in HCT116 cells containing pLXSNU6/H1p53.

C.

24h 5-FU treatment



48h 5-FU treatment



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Figure 14. Overview of the 5-FU genetic selection of spiked siRNA expression libraries.

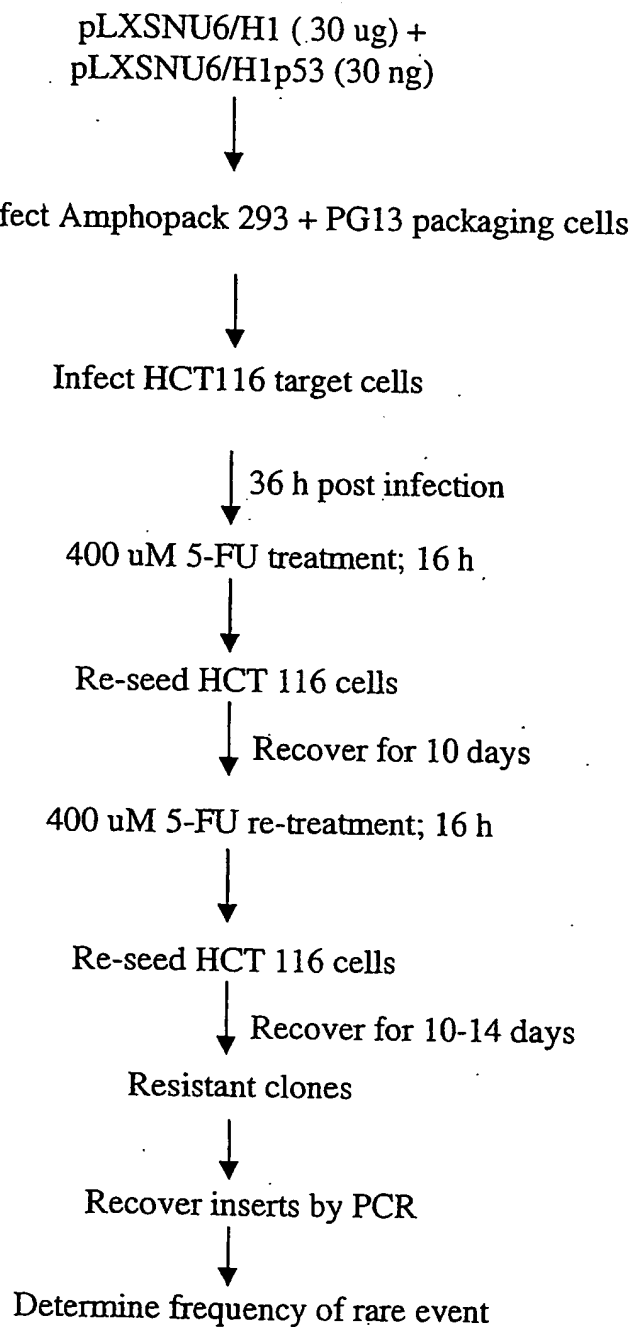


Figure 15. Retroviral expression vectors for genome-wide RNAi libraries.

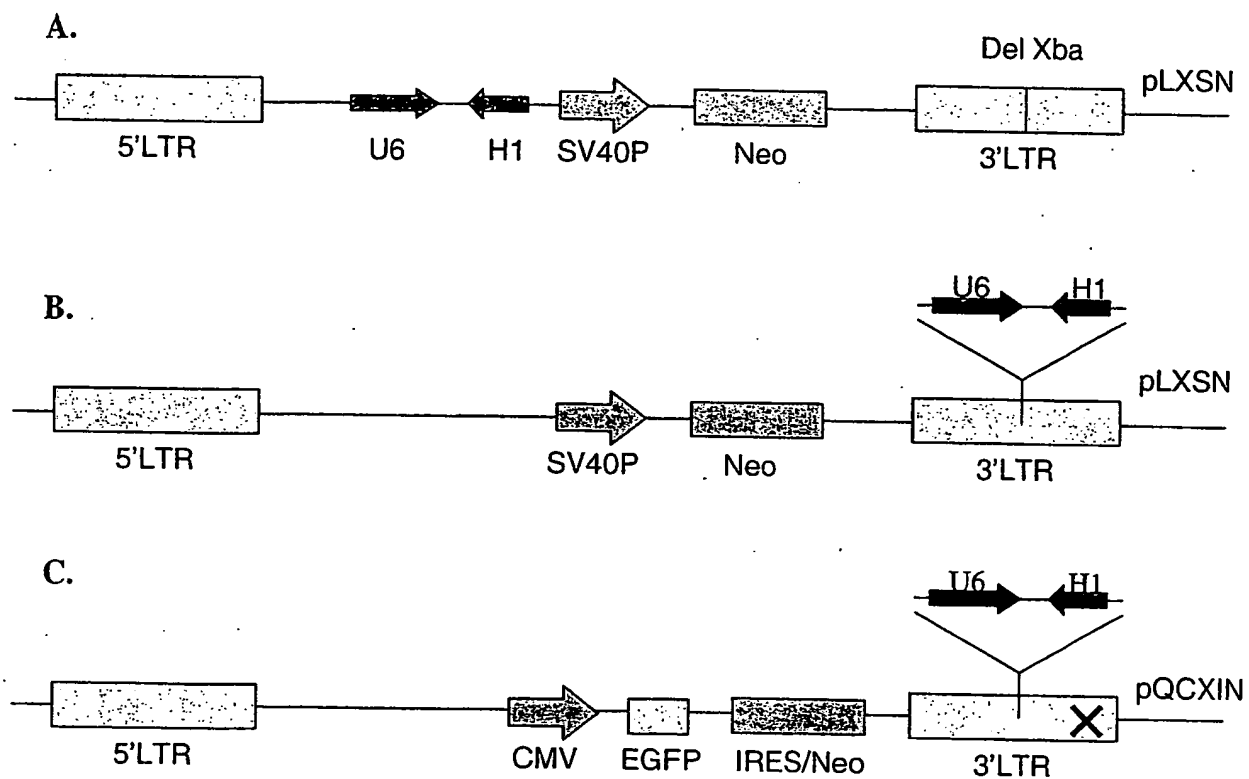


Figure 16. Method for constructing genome-specific shRNA and siRNA libraries

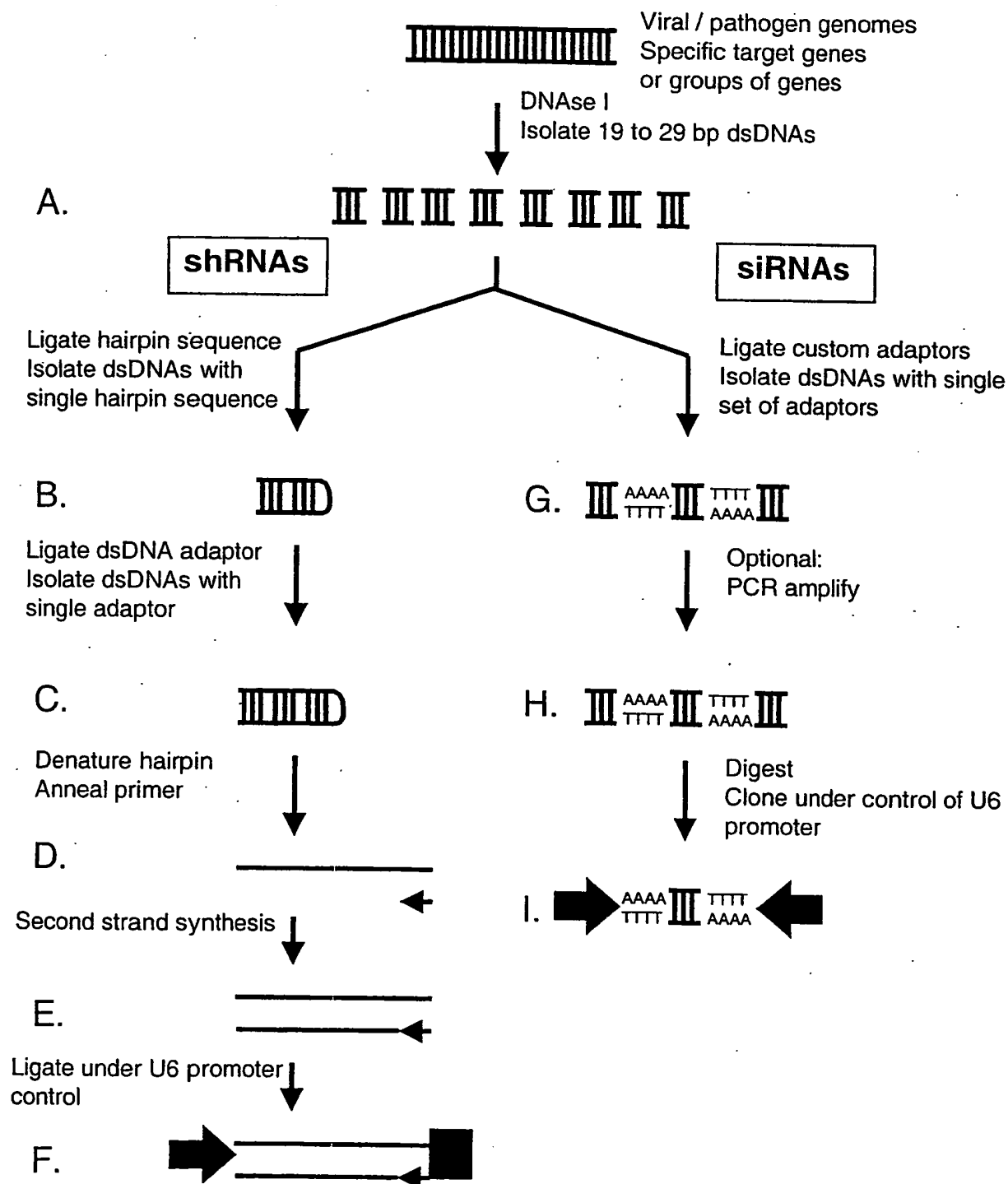


Figure 17. Schematic overview of the method for constructing shRNA and siRNA libraries specific for an expressed RNA population

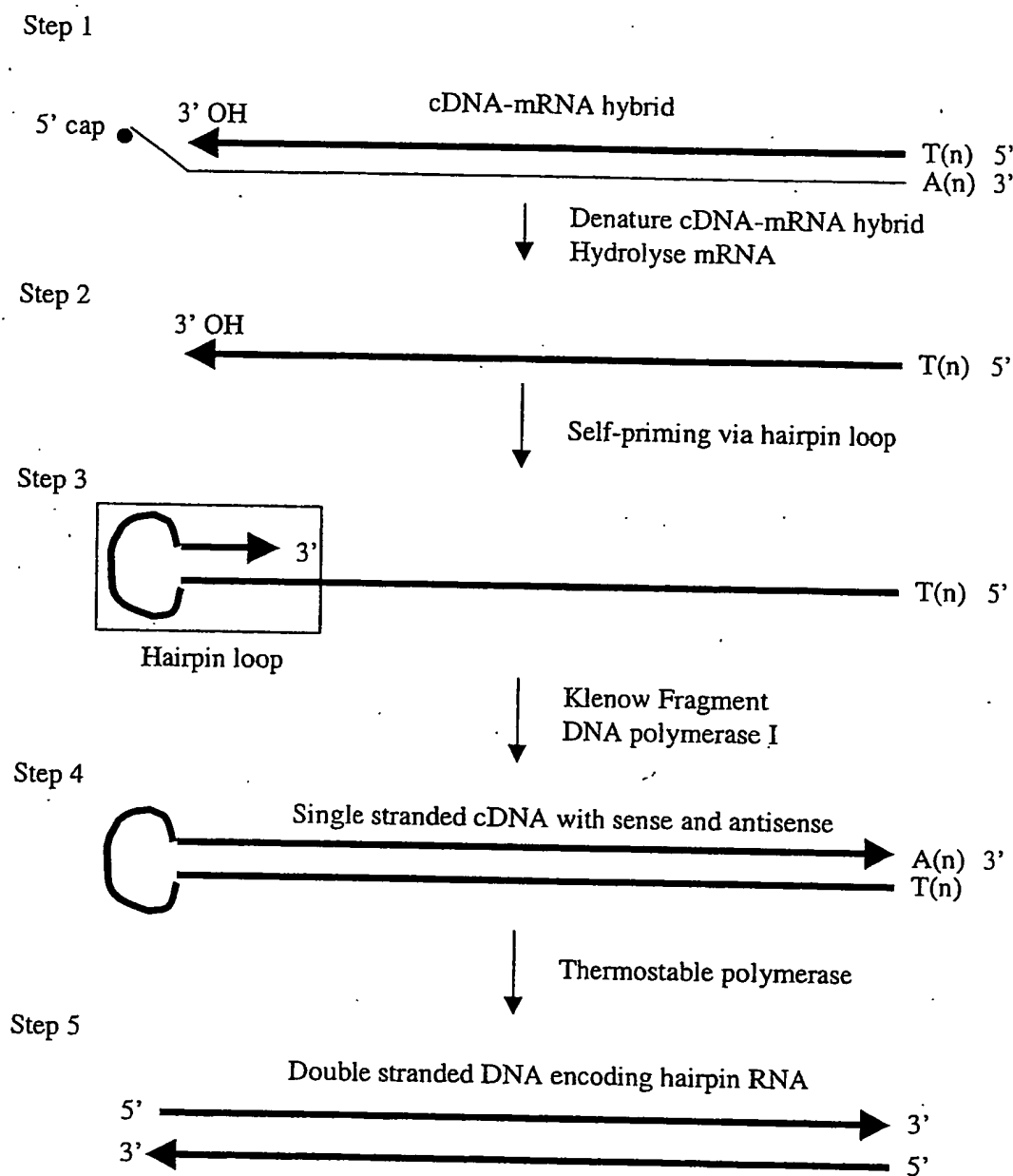


Figure 18. Identification of HIV-specific shRNA or siRNAs using genetic selections

A. Genetic selection for HIV-specific shRNAs blocking induction of HIV

Transfect universal RNAi retroviral expression library into producer cells



Transduce chronically infected promyelocytic clone of HL60



Induce with TNF- α and sort for CD4 positive cells



Purify genomic DNA, PCR amplify shRNA inserts and sequence



Re-test independent shRNA constructs

B. Genetic selection for HIV-specific shRNAs blocking productive infection by HIV

Transfect universal RNAi retroviral expression library into producer cells



Transfect CEM T4 cells



Infect with HIV-1IIIB and sort for CD4 positive and p24 negative cells



Purify genomic DNA, PCR amplify shRNA inserts and sequence



Re-test independent shRNA constructs

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